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Fate of Erythromycin in Sediment-Containing Surface Water Microcosms: How Does Aged Erythromycin in Sediment Influence Bioavailability?

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Abstract
The detection of antibiotics in water and sediment systems is of concern due to the potential adverse effects which could be associated with their environmental fate. The central aim of this study was to evaluate the fate of erythromycin in microcosms consisting of pond water and submerged pond sediment. The first study examined the dissipation of erythromycin from spiked water and total recovery of [14C]-erythromycin from water and sediment within microcosms ranged between 90.1% and 48% throughout the 63-day study. Erythromycin was reduced in surface water of sediment-containing systems by day 7, which corresponded to an increase of eryrthromycin detected in sediment. In the second study the availability of aged erythromycin was evaluated by incubating sediment with and without a manure amendment with [14C]-erythromycin for 0, 1, 3, or 8 weeks; followed by assessing movement and availability of erythromycin in sediment microcosms after 1, 3, 7, and 14 days. Results indicated differences in residues from aged sediment, with and without manure additions, in extractable residues at day 7 and 14. The addition of manure resulted in greater extractable erythromycin from aged sediments than from sediments without manure. There was a greater release of erythromycin to the water overlying the manure-treated sediments with fresh and 1 week aged sediment than the unamended sediment after 1 and 2 weeks. The results from this experiment demonstrate the ability of manure to influence the fate of erythromycin in environmental matrices.

Keywords
Erythromycin, sediment-containing systems, manure, microcosms

Comments
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Chapter 7

Fate of Erythromycin in Sediment-Containing Surface Water Microcosms: How Does Aged Erythromycin in Sediment Influence Bioavailability?

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The detection of antibiotics in water and sediment systems is of concern due to the potential adverse effects which could be associated with their environmental fate. The central aim of this study was to evaluate the fate of erythromycin in microcosms consisting of pond water and submerged pond sediment. The first study examined the dissipation of erythromycin from spiked water and total recovery of [^{14}C]-erythromycin from water and sediment within microcosms ranged between 90.1% and 48% throughout the 63-day study. Erythromycin was reduced in surface water of sediment-containing systems by day 7, which corresponded to an increase of eryrthromycin detected in sediment. In the second study the availability of aged erythromycin was evaluated by incubating sediment with and without a manure amendment with [^{14}C]-erythromycin for 0, 1, 3, or 8 weeks; followed by assessing movement and availability of erythromycin in sediment microcosms after 1, 3, 7, and 14 days. Results indicated differences in residues from aged sediment, with and without manure additions, in extractable residues at day 7 and 14. The addition of manure resulted in greater extractable erythromycin from aged sediments than from sediments without manure. There was a greater release of erythromycin to the water overlying the manure-treated

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Introduction

Antibiotics continue to be an emerging contaminant of concern due to their increase in usage and detection in the environment. Various classes of antibiotics have been found in environmental sampling studies, and concentrations have been measured in a broad range within various matrices including water, soil, sediment, and manure (*1–9*). The presence of these compounds in the environment could potentially affect many aspects of ecosystem function including alteration of bacterial populations leading to nutrient cycle impacts, potential adverse effects to aquatic and non-target organisms, and possibly influence human health (*10*, *11*). One environmental entry point of antibiotics is through land application of manure (*7*, *12*, *13*). Antibiotics are administered to livestock and poultry for treatment of infections as well as for disease prevention, growth promotion, and feed efficiency (*14*, *15*). The majority of the antibiotics administered to agricultural animals are excreted as parent compound, due to low absorption rates (*16*, *17*). Manure produced from these animals is ultimately applied to farmland by injection or waste incorporation as fertilizer to improve crop growth and development (*4*, *16*, *18*, *19*). Detection of antibiotics in water, sediment, soil, and manure samples has been prevalent over the past few years, with tetracyclines, sulfonamides, and macrolides being the most frequently detected antimicrobial compounds.

Macrolides, including erythromycin and tylosin, are one of the most frequently detected antibiotic classes in the environment. Erythromycin's structure is comprised of a 14-member lactone ring with two sugar groups with a molecular weight of 733.9, a pK_a of 8.8 and a K_{ow} of 3.06 (*20–23*). This antibiotic is effective against most gram-positive and some gram-negative bacteria, and its mode of action is through blocking elongation of peptide chains in the ribosome, which inhibits protein synthesis (*21*, *24*, *25*). Elimination of erythromycin occurs through bile and feces at a rate of 50-67% and with urinary excretion at 5-10% (*26*). The high excretion rates of erythromycin may allow for environmental entry of the compound through manure application to agricultural fields, which could enter water and sediment systems through runoff events.

The United States Geological Survey in a 2002 study found 48% of 139 streams tested contained antibiotics, and the second most frequently detected antibiotic from this study was erythromycin (*2*). Another study conducted in 2002 detected antibiotics in 31% of samples collected near swine farms and 67% of samples near poultry farms, with tetracyclines and macrolides (e.g.

erythromycin, tylosin) having the highest concentrations (*3*). One of the most prevalent macrolide antibiotics detected in water samples has been erythromycin, ranging in concentration between 50 ng L^{-1} and 300 ng L^{-1} (*2*, *3*).

In addition to the detection of antibiotics in surface waters, these compounds have also been found in sediment systems and manure slurries. Macrolides have been found in sediment samples with reported concentrations ranging between 2.1 μg kg^{-1} to 24.3 μg kg^{-1} (*5*). Erythromycin was detected in sediment samples ranging between 82 μg kg^{-1} to 128 μg kg^{-1}, which are markedly higher concentrations compared to water systems (50 ng L^{-1} to 300 ng L^{-1}), possibly due to the amount of aged residues that are sequestered within the sediments (*4–6*, *27*). In an environmental monitoring study erythromycin was determined to have the highest relative loss with greater than 50% loss due to runoff and erosion in a rainfall study compared to other antibiotics examined (*6*). The half-life of erythromycin in soil has been experimentally determined to be between 11.5 and 20 days (*7*, *28*). Another macrolide antibiotic found to have strong sorption in soils was tylosin, which demonstrated an affinity to adsorb to manure and sediments, as evidenced by recoveries of less than 2.5% from soil columns (*9*). It has been suggested that macrolide antibiotics, including erythromycin and tylosin, are adsorbed to clay particles, organic matter, or manure in soil, which reduce their degradation and leaching (*12*). These studies found macrolide antibiotics in multiple environmental matrices, with tylosin and erythromycin being widely detected. However, less information regarding erythromycin's environmental fate is available compared to tylosin and further research is needed to understand its behavior within the environment.

Erythromycin has the potential for transport in the environment through runoff from manure-treated fields leading to erythromycin's entry into water, which in turn leads to erythromycin in sediment where it may persist and age, and be bioavailable for uptake by terrestrial and aquatic organisms to some unknown extent Although few studies have focused on aged antibiotic residues in sediments, many studies have examined other organic compounds including herbicides and insecticides (*29–31*). Some herbicides and insecticides bioaccumulate in organisms within sediment and affect non-target organisms, but bioavailability of these aged residues within the environment is influenced by sediment characteristics (particles size, pH, clay content, and organic matter content) which affect adsorption and desorption rates of those compounds (*30*, *32*).

The overall aim of this study was to investigate the fate of erythromycin in a pond water and pond sediment microcosm through examination of its ability to bind to organic particulate matter and sediment, to partition between water and sediment, and of its abiotic and biotic degradation within the environment. This paper examines erythromycin's movement within water and sediment microcosms, specifically to improve the understanding of erythromycin's environmental fate and to simulate the impact of erythromycin run-off, which commonly occurs with manure from agricultural field application. Potential bioavailability of erythromycin residues in sediment were examined after aging for 0, 1, 3, or 8 weeks.

Materials and Methods

Chemicals

Acetonitrile (HPLC grade), acetic acid, ammonium acetate, sodium hydroxide, erythromycin, ashless cellulose powder, and Ultima Gold scintillation cocktail were purchased from Fisher Scientific (Pittsburgh, PA). Carbosorb E and Permafluor E+ scintillation cocktails were purchased from Perkin and Elmer (Waltham, MA). [^{14}C]-radiolabeled erythromycin was purchased from American Radiolabeled Chemicals (St. Louis, MO). The [^{14}C]-label was present in one of the methyl groups bonded to the nitrogen of the desosamine sugar of the erythromycin molecule.

Pond Water, Pond Sediment, and Manure Collection

Pond water and sediment were collected from the Iowa State University Horticulture Research Station (Gilbert, Iowa). Sediment was manually collected by inserting a soil auger 10 – 15 cm (depth) into the pond sediment. Sediment composition was determined as 60 % sand, 28 % silt, 12% clay, 2 % organic matter, and a pH of 8.1. Water had an alkalinity of 103 mg ml^{-1} and total hardness was 150 mg ml^{-1}. Sediment moisture was 47% prior to use. Water and sediment samples were transported to the lab and were stored at 4° C until use (< 7 days). Fresh manure was obtained from the Iowa State University Swine Nutrition Farm (Iowa State University) from antibiotic-free pigs on a corn-soybean-based diet. Manure characterization was completed by the Iowa State University Agricultural Waste Management Laboratory indicating a pH of 6.3 and containing: 33.6% total solids, 1.7% total Kjeldahl nitrogen, 1.1% total phosphorous,0.7% ammonia, and 0.4% dissolved reactive phosphorous. The collected manure was kept at 4°C until use (< 7 days).

Environmental Fate Experimental Design and Analysis

This study examined the fate of erythromycin entering a simulated pond in runoff from an upstream source. Four different microcosm treatments: pond water only (PW), pond water overlying pond sediment (PWS), autoclaved pond water overlying autoclaved pond sediment (APWS), and pond water with dilute swine manure overlying pond sediment (PWS+M). The APWS treatment aimed to measure sorption and non-biotic processes, while the PWS treatment focused on the combined impact of sediment sorption and biodegradation, and the PWS+M examined the impact of manure associated with runoff. The PW treatment was used to assess the impact of erythromycin degradation in water. All treatments utilized in this study were selected to investigate erythromycin's potential environmental fate and their detection in matrices (water, sediment, and manure), with the APWS treatment serving as a control.

One week prior to set-up 4 L of pond water and 1200 g of pond sediment were autoclaved three times at 121°C in one-hour cycles for use in the APWS treatment at one-day intervals. The PW treatment consisted of 200 ml of pond water, while the APWS treatment had 64.8 g (50 g dry wt.) autoclaved pond sediment and 185.2

ml autoclaved pond water. For the PWS and PWS+M treatments the microcosm was comprised of 73.5 g (50 g dry wt.) pond sediment and 176.5 ml pond water. Microcosms were assembled in wide-mouth 470-ml jars (Ball Corp., Broomfield, CO), 50 g dry weight of sediment and 200 ml pond water per jar and were incubated for 7, 14, 28, or 63 days. Each jar served as a replicate with four replicates per treatment and timepoint.

Sediment was allowed to settle one hour prior to [^{14}C]-erythromycin addition. The treatment solution utilized in this study was comprised of labeled and non-labeled erythromycin which was added to each treatment replicate. Treatment spiking solution was prepared with 85 mg of non-labeled erythromycin to obtain a concentration of 0.425 mg ml^{-1} in a 200 ml volumetric flask and 171 µl of 0.1 mCi ^{14}C-radiolabeled erythromycin (specific activity of 55 µCi mmol^{-1}). Each treatment replicate received 2.35 ml of the treatment spiking solution yielding final concentrations of 5 mg L^{-1} and 0.201 µCi per jar.

For the PWS+M treatment a manure slurry was prepared by adding 33 g of manure to 100 ml distilled water to get a 33% slurry solution. The slurry was stirred for 40 minutes to break up large chunks, and 0.6 ml of slurry was added to each replicate giving the treatments a murky appearance compared to the treatments without the manure amendment. The autoclaved treatment was assembled and analyzed in a laminar flow hood using sterile equipment to maintain sterile conditions. All treatments were maintained in a 24° C environmental chamber with a 12:12 photoperiod. The pH of water in all treatments was monitored weekly and did not vary significantly throughout the course of the study.

Mineralization of [^{14}C]-erythromycin was tracked throughout the study by using sodium hydroxide solution traps for CO_2 evolution. A 25-ml high-density polyethylene vial was glued onto the inner surface of each jar and was filled with 10 ml of 0.5 M sodium hydroxide. Traps were changed on Day 3, 7, 14, 21, 28, 35, 42, 49, and 56 of the study. Three milliliters of each sodium hydroxide sample was mixed with 12 ml Ultima Gold cocktail, mixed, and was counted for radioactivity on a Beckman Coulter 6500 liquid scintillation counter ((LSC), Fullerton, CA).

After 7, 14, 28, and 63 days of incubation the distribution of [^{14}C] in water and sediment was determined. Treatment water was removed from each replicate jar and [^{14}C]-erythromycin radioactivity was counted on the LSC using 1 ml of water with 15 ml Ultima Gold cocktail. Next, the water samples were filtered through 0.2-µm, 47-mm diameter nylon filters (Fisher Scientific). Following filtering, water samples were extracted using Oasis® HLB cartridges (6 cc, Waters Corp., Milford, MA). After extraction of water samples, radioactivity was assessed with 1 ml of water and 15 ml Ulotima Gold cocktail . Cartridges were conditioned using the Kolz et al., (2006) solid phase extraction method. Recovery of ^{14}C-residues of applied [^{14}C]-erythromycin was determined to be 94.7% ± 4.9 from pond water utilizing this method.

Sediment was extracted with 100 ml of acetonitrile: 0.3 M ammonium acetate at pH 4.2 (85:15, v/v), and each sample was shaken on an orbital shaker for 85 minutes at 300 rpm. Samples were allowed to settle overnight at room temperature followed by siphoning off the liquid extract. A second 100-ml aliquot of acetonitrile: 0.3 M ammonium acetate at pH 4.2 (85:15, v/v) was

added to each sediment sample and shaken on an orbital shaker for 15 minutes at 300 rpm followed by centrifuging and decanting. Each sediment extract sample was concentrated to a volume of 1 ml under nitrogen flow at 15 psi, at 50°C and reconstituted to a final volume of 10-ml with acetonitrile. A 3-ml aliquot of sediment extract for each sample was mixed with 12 ml Ultima Gold cocktail and counted for radioactivity on the LSC. Extracted sediment samples were allowed to dry in a fume hood for 24 hours. Dried sediment was sieved through a 5-mm sieve, followed by a 2.5-mm sieve to remove any large non-combustible material. Sieved sediment samples were ground using a mortar and pestle. Next, sediment pellets were constructed with 0.5 g dried, ground sediment and 0.5 g ashless cellulose powder (1:1 ratio). Sediment pellets were oxidized using a Packard Model 307 oxidizer (Perkin Elmer, Waltham, MA) with a two-minute combustion time. Following oxidation, sediment sample vials containing reagents were counted for radioactivity on the LSC to determine bound [^{14}C]-erythromycin residues.

Aged Sediment Experimental Design

Two metal pans were filled with 2.87 kg of pond sediment, and to one of the pans a manure slurry was then added. The manure slurry contained 57.4 g of manure dissolved in 50 ml of distilled water and was stirred for 50 minutes until thoroughly mixed, followed by addition to one container of sediment. Treatment spiking solution was prepared with 24.2 mg of non-labeled erythromycin to obtain a concentration of 0.121 mg ml^{-1} in a 200 ml volumetric flask and 303 µl of 0.1 mCi ^{14}C-radiolabeled erythromycin (specific activity of 55 µCi mmol^{-1}). Each treatment replicate received 97.5 ml of the treatment spiking solution yielding final concentrations of 3.775 mg L^{-1} and 0.19 µCi per jar.

Erythromycin residues in the sediment were aged for 0, 1, 3, or 8 weeks prior to microcosm assembly and were kept at 25°C using a 16:8 light:dark photoperiod. Microcosms were assembled after the designated timepoints which included 36.75 g (25 g dry weight) of sediment (either with or without manure amendment), and they were topped with 88.25 ml of distilled water in a 250-ml French square bottle. Microcosms were incubated for 0, 1, 3, 7, or 14 days and were performed in replicates of four (n=4). All aged sediment and water columns were maintained in a 25° C environmental chamber with a 16:8 photoperiod.

Water column replicates were sacrificed at the specified timepoints, at which water was removed from treatment containers and SPE was performed as discussed in the environmental fate experimental design and analysis section. Next, sediment was extracted using 50 ml acetonitrile: 0.3 M ammonium acetate at pH 4.2 (85:15, v/v) followed by bound residue analysis with the protocols outlined in the previous experiment environmental fate experimental design and analysis section.

Statistical Analysis

Statistical analysis was performed using SigmaStat 3.0 (Chicago, IL) employing ANOVA analysis with Bonferroni or Dunn's analysis to compare data treatments and time points. Significance level was determined as $P \leq 0.05$

for all analyses. Linear regression and least squares analysis were conducted with SigmaPlot 10 (Chicago, IL) to determine dissipation kinetics in water from treatment samples.

Table 1. Mass balance of [^{14}C]-erythromycin residues in treatment microcosm components[1]

Treatment	*Microcosm System Component*	*Day[2]* 7	14	28	63
PW[a]	Treatment Water	87.5 ± 0.7	89 ± 1.8	80.7 ± 1.1	86 ± 0.9
	Mineralization	0.2 ± 0.03	1.1 ± 0.1	1.3 ± 0.05	2.2 ± 0.02
	Total Recovery	87.7	90.1	82.5	88.2
APWS[b]	Treatment Water	33.7 ± 4.4	24.6 ± 1.0	13.9 ± 2.3	10.5 ± 1.3
	Sediment - Extractable	30.1 ± 2.5	29.2 ± 1.7	32.3 ± 1.2	38.3 ± 6.6
	Sediment - Bound	10.6 ± 0.2	12.2 ± 0.8	13.5 ± 1.1	14.2 ± 0.5
	Mineralization	0.06 ± 0.06	0.2 ± 0.03	0.6 ± 0.02	1.1 ± 0.01
	Total Recovery	74.5	66.2	60.3	64.1
PWS[c]	Treatment Water	19.3 ± 1.6	18 ± 1.0	15.4 ± 1.8	2.6 ± 0.2
	Sediment - Extractable	40.2 ± 3.7	30.7 ± 4.5	12.7 ± 1.3	3.7 ± 0.6
	Sediment - Bound	13.1 ± 0.5	16.4 ± 0.8	20.9 ± 2.0	38.2 ± 1.6
	Mineralization	0.2 ± 0.02	1.8 ± 0.3	12.6 ± 0.9	16.1 ± 0.04
	Total Recovery	72.8	66.9	61.6	60.6
PWS+M[d]	Treatment Water	27.5 ± 3.4	24.6 ± 0.3	9.6 ± 0.8	3.7 ± 0.1
	Sediment - Extractable	38.3 ± 5.6	36.1 ± 2.1	9.1 ± 0.2	4.4 ± 0.4
	Sediment - Bound	11.3 ± 0.3	16.1 ± 0.9	32.3 ± 2.6	28.7 ± 1.2
	Mineralization	0.2 ± 0.02	1.3 ± 0.2	8.8 ± 0.6	11.2 ± 0.07
	Total Recovery	77.3	78.1	59.8	48

[1] Values shown are mean percentage of applied radioactivity ± standard error. [2] Day post addition of [^{14}C]-erythromycin added to water portion of microcosm. [a] Pond Water. [b] Autoclaved Pond Water and Autoclaved Pond Sediment. [c] Pond Water and Pond Sediment. [d] Pond Water with Manure Slurry and Pond Sediment.

Results and Discussion

Freshwater Microcosm Study: Mass Balance

Mean percentages of [^{14}C]-residues recovered 63 days after [^{14}C]-erythromycin application to experimental microcosms are listed in Table 1. The pond water (PW) treatment recovery ranged between 89.9% and 81% throughout the course of the study. The APWS treatment displayed a decrease in mean [^{14}C] total recovery throughout the course of the study except with a small increase from day 28 to 63. The APWS water showed a decrease in [^{14}C]-erythromycin recovery and a slight increase in extractable and bound sediment residues. For the PWS treatment, a decrease in total recoverable mean [^{14}C]-erythromycin between day 7 and 63 occurred, 72.8% to 60.6%. Microcosm components for the PWS treatment displayed a decrease in radioactive residues for treatment water and extractable sediment residues, but an increase with sediment bound residues. The PWS+M treatment displayed similar [^{14}C]-erythromycin residue patterns in all microcosm components to the PWS treatment. ANOVA analysis indicated significant differences between the total recovery in the treatments examined ($p = 0.034$).

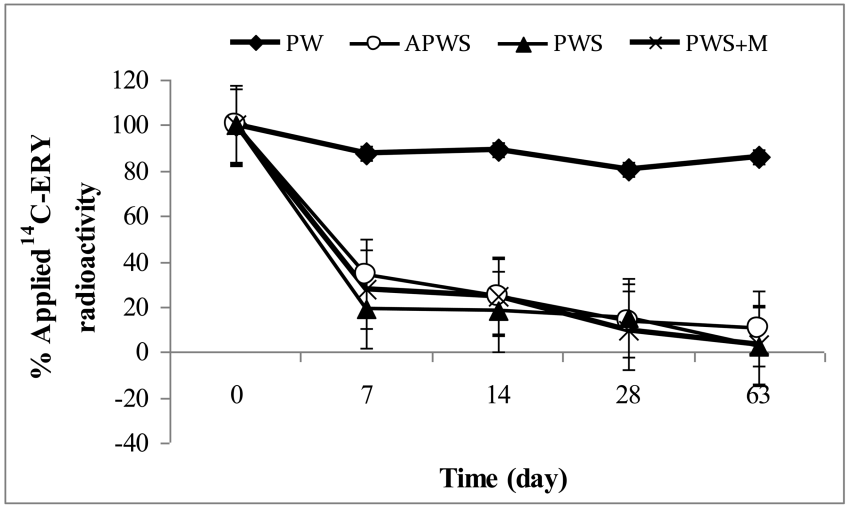

Figure 1. Percentage of [^{14}C]-ERY remaining in surface water.

Freshwater Microcosm Study: Dissipation Kinetics

[^{14}C]-erythromycin residues in surface water remained fairly constant in the PW treatment throughout the study (Figure 1). The PW treatment was significantly different in the quantity of [^{14}C] residue in surface water compared to the sediment-containing systems, with a greater amount present in the PW treatment compared to all other treatments examined. In treatments APWS, PWS, and PWS+M a sharp

decrease in [^{14}C]-erythromycin was noted between day 0 and day 7, with 33% to 19% remaining in water by day 7 and continued to decrease by day 63 with <10% remaining in the water portion of the microcosm. The pH of the water throughout the course of the study did not vary greatly between day 0 and 63.

Dissipation kinetics in water were examined, and results indicated that erythromycin dissipates from water via a one-compartment model for the PWS and PWS+M treatments. (Equation 1). However, model was not valid for the PW and APWS treatments as poor correlation was observed. A DT_{50}, the time for the concentration of a chemical to reach 50% of applied, was also calculated for the dissipation of erythromycin for the treatments examined, using Equation 2 for all treatments.

$$(1)\ C = C_0 e^{(-k_t)}$$

$$(2)\ DT_{50} = 0.693/k$$

The variables used in the equations above represent the following:
C = erythromycin concentration at time t
C_0 = initial erythromycin concentration
k = first-order rate constant for erythromycin
t = time (days)

Table 2 lists the calculated parameters for the model shown above. Erythromycin dissipates from water to 50% of the applied dose by 5.8 days for APWS and PWS treatments, while it takes 5 days for the PWS+M treatment. The first-order, one-compartment model was used for the APWS, PWS and PWS+M treatments with r^2 values greater than 0.7 and this model has been utilized in pesticide risk assessment (*33*). However, with these treatments better r^2 values were obtained with a three-parameter modified single, exponential decay model for the treatments but these models do not have a mechanistic interpretation as do first-order or two-compartment dissipation kinetic models.

Table 2. Dissipation kinetics for erythromycin in treatment water of surface water microcosm systems

Treatment	*F value*	*Dissipation Model*	*k*	*r^2*	*p-value*	*DT_{50} (days)*
PW	1.9088	$C=C_0e^{(-kt)}$	0.0019	0.32	0.32	—
APWS	44.73	$C=C_0e^{(-kt)}$	0.1187	0.9986	0.007	5.8
PWS	44.73	$C=C_0e^{(-kt)}$	0.1895	0.9891	0.007	5.8
PWS+M	61.54	$C=C_0e^{(-kt)}$	0.1386	0.9889	0.0043	5

The dissipation of erythromycin from water is mostly due to its partitioning into sediment in the APWS, PWS, and PWS+M treatments. The total recovery of [^{14}C]-residues was examined to determine when the microcosm treatments containing sediment reached 50% of applied by plotting the $\log_{10}$ of total percentage recovered in microcosms at 7, 14, 28, and 63 days versus time and fitting a linear trendline to obtain treatment specific equations listed in Table 3. Results indicate that the DT_{50} of 38 days for the PWS+M treatment is shorter than the 45 day DT50 for PWS treatment. However, the important role microorganisms have in degrading and utilizing erythromycin in the environment may influence erythromycin degradation.

Table 3. Equations for calculating DT_{50} remaining [14C]-applied within sediment containing microcosm treatments including days when 50% is reached

Treatment	*Linear Trendline Equation*	r^2	*DT_{50} of [^{14}C] remaining in microcosm (days)*
APWS	y = -0.0009x + 1.8438	0.3675	—
PWS	y = -0.0037x + 1.8692	0.91	45
PWS+M	y = -0.0057x + 1.9189	0.93	38

Mineralization of Erythromycin in Freshwater Microcosm

The inclusion of sediment increased $^{14}CO_2$ evolution from mineralization in pond water (Figure 2). The PWS and PWS+M treatments displayed similar trends in CO_2 evolution with a lag phase between days 0 and 7, followed by an exponential growth phase between days 7 and 35. After day 35, $^{14}CO_2$ in these treatments began to plateau through day 63. The PWS+M treatment had less mineralization compared to the PWS treatment in total amounts of $^{14}CO_2$ evolved throughout the study, with significant differences seen between the APWS compared to PWS and PWS+M ($P = 0.023$). The PW and APWS treatments were similar in the amount of $^{14}CO_2$ evolved, with <2% of applied ^{14}C-radiolabel detected in these treatments.

Mineralization is a common microbial process and the amount of mineralization occurring in the PWS and PWS+M microcosms may indicate a wide distribution of erythromycin-degrading microorganisms within the pond sediment. This distribution may be due to an increase in the density of microbial populations degrading erythromycin, especially those capable of degrading erythromycin including some gram-negative microorganisms (*34*, *35*). Kim et al., (2004b) demonstrated that the [^{14}C]-radiolabeled methyl group is more readily hydrolyzed compared to [^{14}C]-radiolabeled groups of the macrocyclic lactone ring. The cumulative mineralization rates in this study were 10% to 15%

for PWS+M and PWS treatments, respectively,which were greater than those reported by Kim et al., 2004. Our increased mineralization rates may be due to the position where erythromycin used in our study was labeled.

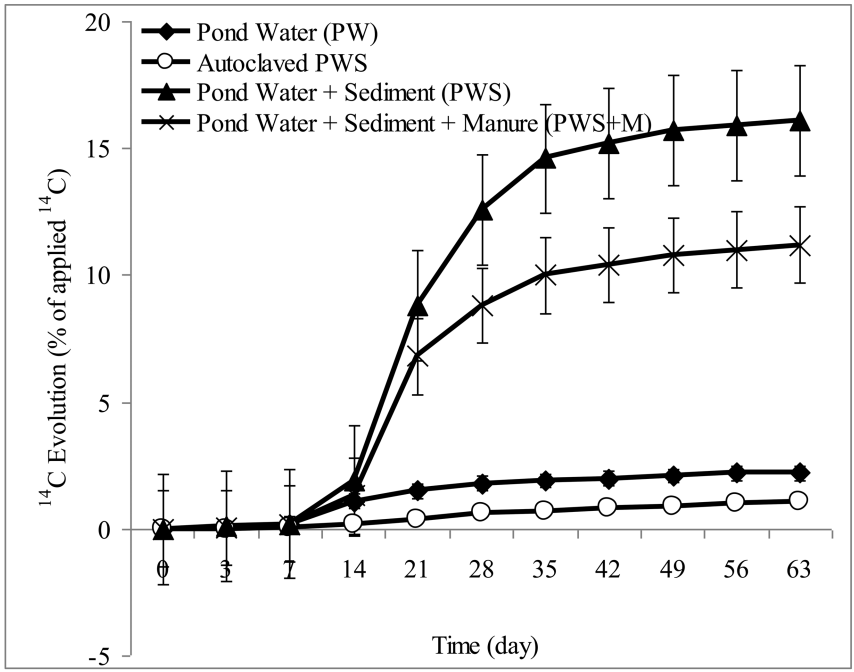

Figure 2. Cumulative mineralization of [^{14}C]-erythromycin from microcosm treatments.

Freshwater Microcosm Study: [^{14}C]-Erythromycin in Sediment

Erythromycin movement into the sediment corresponded to an increase in extractable residues and bound [14C]-residues (Fig. 3). The APWS treatment displayed a plateau of bound [^{14}C]-residues throughout the 63-day study A slight increase in bound residues between day 7 and day 63 was seen in the PWS and PWS+M treatments, most likely due to erythromycin interacting with clay and organic matter causing binding to occur through abiotic processes. In addition, this decrease in extractable residues may be attributed to microorganisms utilizing erythromycin and subsequently incorporating it as biomass. In the PWS and PWS+M treatments extractability of erythromycin decreased between day 7 and day 63. Bound [^{14}C]-residues within PWS demonstrated a linear increase from day 0 to day 63, and a decrease in extractable [^{14}C]-residues between day 7 and day 63. In contrast, the PWS+M treatment showed a slight decrease between day 7 and 14 in extractable erythromycin residues, followed by a sharp reduction between day 14 and 63. Bound residue in the PWS+M treatment increased from day 0 to day 28.

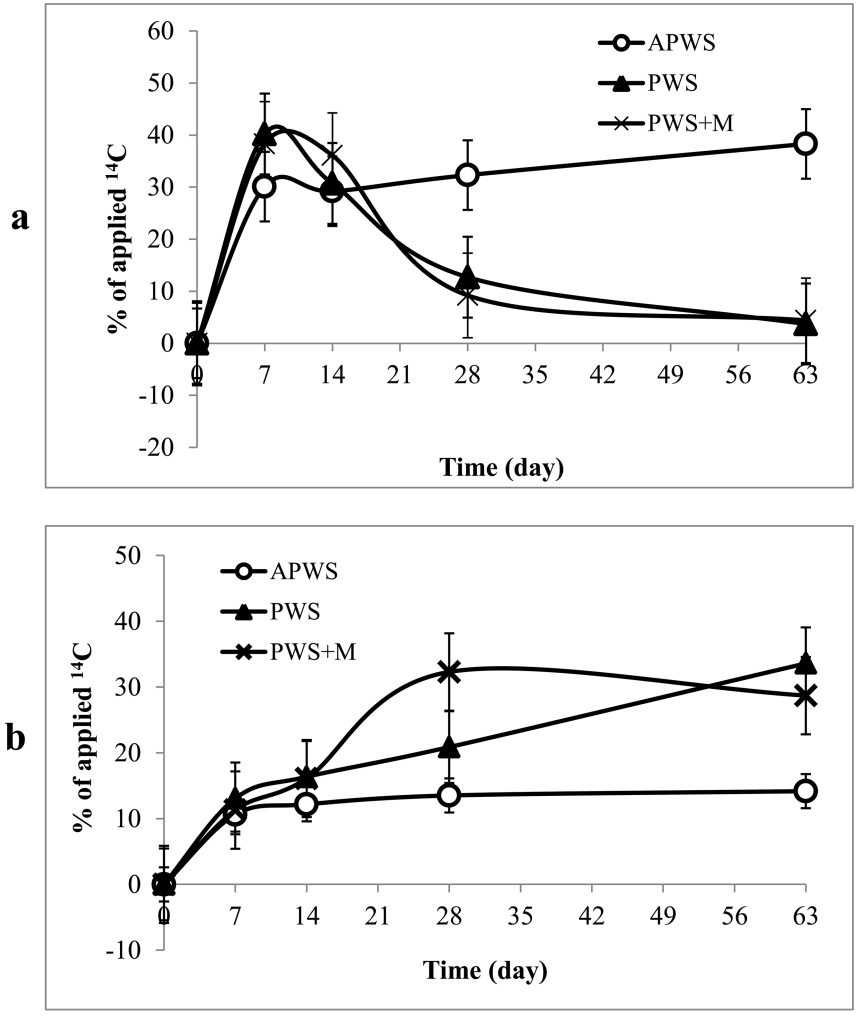

Figure 3. Percentage of extractable and bound [^{14}C]-residues derived from applied [^{14}C]-erythromycin (a) extractable [^{14}C]-residues (b) bound [^{14}C]-residues.

Erythromycin accumulated in sediment with a decrease in extractable residues and an increase in bound residues observed throughout the course of the study in the non-autoclaved treatments. We found 40% to 50% erythromycin in sediment components after 7 days, with slightly more erythromycin in the manure-containing treatment. These studies demonstrate that erythromycin accumulates in sediment with the potential for degradation to occur through biotic processes. However, additional experiments are needed to better understand the degradation pathway and the influence of chemical and biological parameters (pH, temperature, etc…) on sorption.

Aged Residues Study: Sediment

Our results and those of others (*9*, *34*, *36*, *37*) show that erythromycin and other antibiotics partition from the overlying water into stream and pond sediments. We examined the dissipation of erythromycin residues in sediment that had been previously aged for 1, 3 or 8 weeks. Extractability of [^{14}C]-erythromycin from aged sediments was assessed prior to the assembly of the microcosms (Figure 4), and show a decrease over the 1 week, 3 weeks, and 8 weeks of aging, but no difference was seen between the two matrices examined. After microcosms were established and the non-aged (fresh) and aged residues were submerged, the [14C]-erythromycin degraded slowly (Figure 5). There was little change in extractable residues freshly added to sediment over the 14 day period. Residues aged for 1 week and 8 weeks decreased to about 50% of their levels at the start of the incubation, but the 3 week aged residues showed little decline over the 14 day period, similar to the freshly added residues.

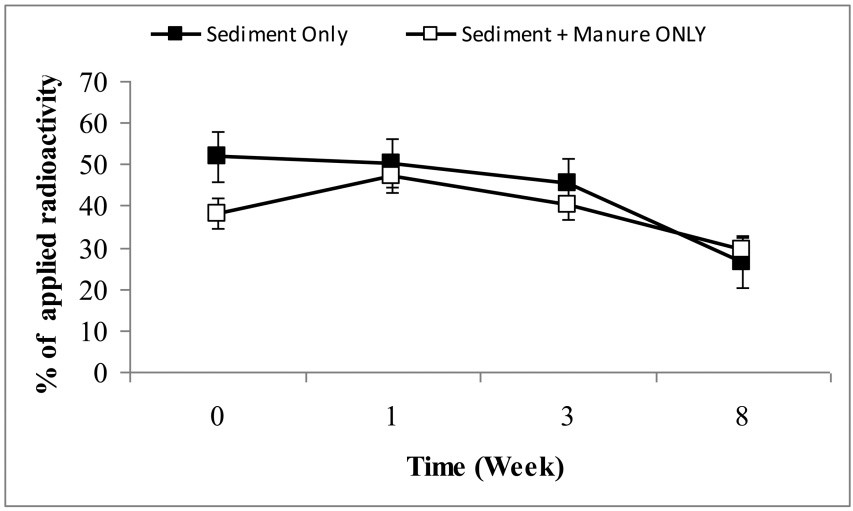

Figure 4. Extractable aged [^{14}C]-erythromycin residues (% of applied [^{14}C]) in sediment matrices with and without manure amendment prior to assembly of surface water columns. The time on the x-axis represents the number of weeks the microcosms containing water, sediment were incubated.

Statistically significant differences were found in extractable residues including fresh sediment compared to 3 and 8-week aged sediment ($P < 0.001$ and $P = 0.008$). Week 1 aged sediment extractable residues were statistically different from week 8 aged sediment extractable residues ($P = 0.029$). No significant differences were seen in sediment with manure amendment at the various time points examined (0, 1, 3, and 8 weeks). Comparison between sediment and sediment with manure amendment treatments aged 0, 1, 3, and 8 weeks showed a significant difference ($P=0.006$). The extractable [^{14}C]-residue results from the aged study are increased compared to the fate study, which may be due to the

incorporation route of the manure and erythromycin in each study. In the aged study the manure was mixed directly into sediment versus the fate study which utilized a manure slurry added to water representing manure runoff from rainfall. The manure incorporation route utilized in this experiment could also represent antibiotics which enter river sediment that is overlain with fresh stream water. This difference in manure and residue environmental entry routes could suggest erythromycin's potential to be more bioavailable in sediment.

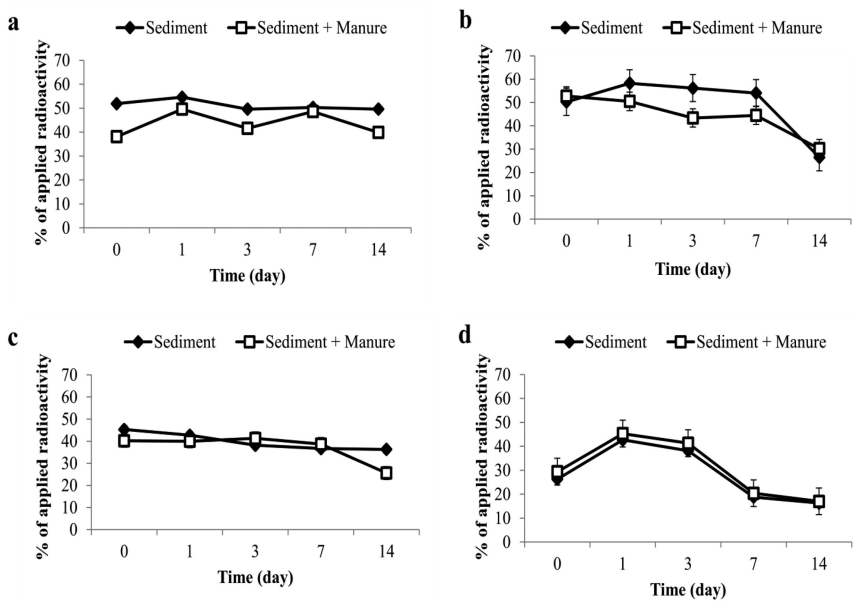

Figure 5. Extractable [^{14}C]-ERY (% of applied [^{14}C]) in sediment and sediment with manure amendment (50:1, v/v) followed by addition of distilled water and incubation for 1, 3, 7, or 14 days; (a) fresh (b) Aged 1 week (c) Aged 3 weeks (d) Aged 8 weeks.

Aged Residues Study: Water

The percentage of [^{14}C]-erythromycin moving into surface water from the aged erythromycin residues in sediment is shown in Figure 6. More [^{14}C]-erythromycin was released into surface water from the manure-containing sediment treatment compared to the sediment only matrix. In the sediment only system, fresh and 3-week-aged [^{14}C]-residues yielded higher amounts of erythromycin in surface water compared to 1 and 8-week aged [^{14}C]-residues by day 14 (Figure 6a). The sediment with manure amendment showed more erythromycin in water in the fresh and 1 week aged treatments at day 14 compared to 3 and 8-week aged samples. No significant differences were seen between aged [^{14}C]-residue treatments in the water component of sediment containing microcosms (without manure) for 1, 3, 7, and 14 days.

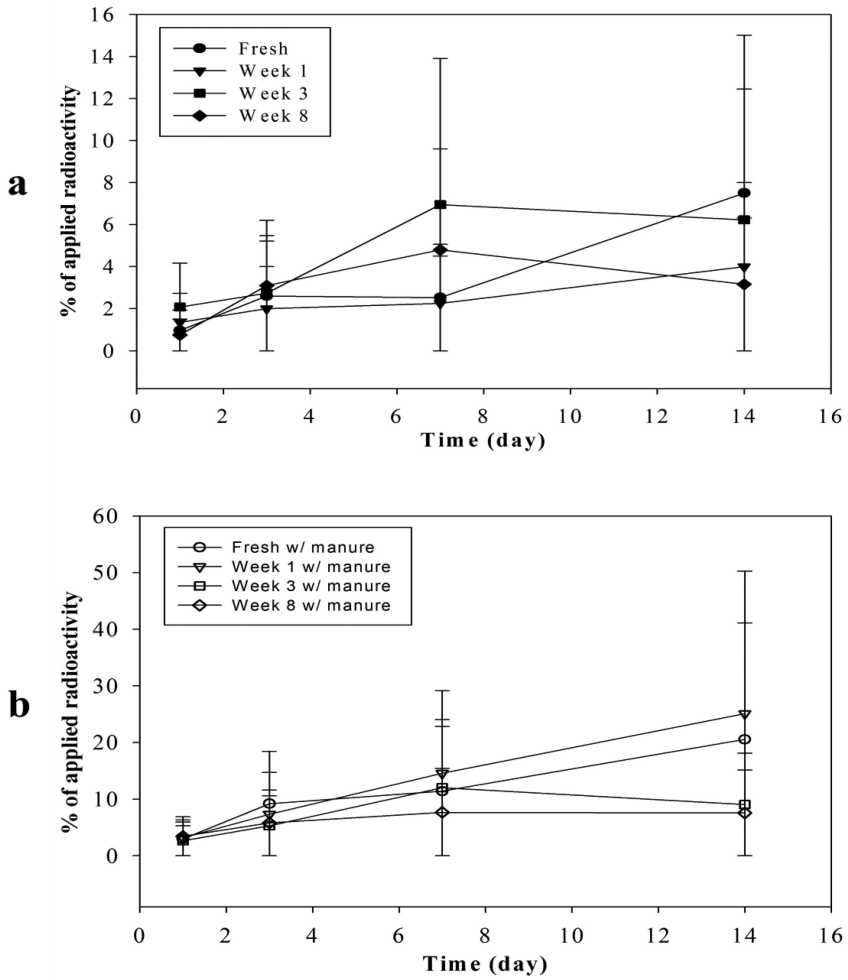

Figure 6. Percentage of [^{14}C]-ERY in surface water released from sediments treated with [^{14}C]-erythromycin at zero (fresh), 1, 3, and 8 weeks previously (a) Pond sediment system (b) Pond sediment + manure (50:1, v/v by weight) system.

Addition of manure to sediment influenced the availability of [^{14}C]-erythromycin residues in surface water with greater amounts seen in the water portion for fresh and 1-week aged treatments. Overall, aged residues in sediment with manure showed an increase in [^{14}C]-erythromycin into water between day 0 and 7 for all treatments (Figure 6b). A statistically significant difference was observed with erythromycin released from aged residues in sediment at 7 and 14 day incubation times with surface water ($P = 0.021$; $P = < 0.001$). By day 14 a decrease in erythromycin from water was seen with 3 and 8-week aged sediment with manure amendment, compared to an increase in fresh and 1 week aged treatments. Examination of extractable ^{14}C-residues from sediment with

manure amendment incubated for 0, 1, 3, and 8 weeks indicated no significant difference between surface water incubation timepoints (1, 3, 7, and 14 days) with ANOVA analysis (P = 0.08). Statistical analysis indicated that there is a significant difference between aged erythromycin in sediment with manure after 7 and 14 days of water incubation in fresh and 1-week aged compared to 3 and 8-week aged treatments (P = < 0.001; P = < 0.001).

Conclusions

Erythromycin dissipates slowly in surface water half-life in the pond water of 365 days compared to the water with underlying sediment (APWS, PWS, and PWS+M) with 5 to 6 day half-lives. The quick dissipation in water is due to the rapid partition of erythromycin into the sediment. In contrast, when the total erythromycin residues in the microcosm are considered, the time for 50% erythromycin loss was 38 to 45 days in non-autoclaved treatments, demonstrating the tendency of sediment to sequester erythromycin. Biodegradation of erythromycin in sediment systems was observed as increased $^{14}CO_2$ evolution in non-autoclaved sediment-containing systems. The PWS and PWS+M treatments displayed higher mineralization rates compared to PW and APWS treatments due to microorganisms present in the sediment.

Erythromycin partitioned back into water from aged sediment, with manure influencing the partitioning in fresh and 1-week aged sediment samples. Erythromycin was found to be extractable from aged sediment and manure-containing sediment samples with a slight decrease observed with an increase in water incubation time. Further studies are needed to understand the bioavailability of erythromycin in the environment to non-target organisms due to this compound adsorbing into sediment and also movement into water from aged residues in sediment. Additional studies to better recognize the potential for metabolites to form in environmental components are needed, which may aid in a better understanding of erythromycin's fate in water and sediment.

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